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Klotho, FGF23, and FGF receptors in chronic kidney disease: a yin–yang situation?

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Secondary hyperparathyroidism in chronic kidney disease (CKD) develops in response to disturbances in calcium and phosphate metabolism associated with CKD, including FGF23 and klotho. FGF23 activates its receptor FGFR1, splice variant IIIC, in the parathyroid gland via a klotho-dependent mechanism and suppresses parathyroid hormone (PTH) secretion. Klotho also may regulate PTH secretion in an FGF23-independent mode, by modulating parathyroid Na^+/K^+-ATPase activity. The persistence of hyperparathyroidism with progressing CKD despite high serum FGF23 is indicative of FGF23 resistance.

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The excessive synthesis and secretion of parathyroid hormone (PTH) in patients with advanced stages of chronic kidney disease (CKD) and the secondary hyperparathyroidism that ensues remain a vexing problem. This is particularly true for those patients who develop a severe form of the disease. For many years, the factors held mainly responsible for the increase in PTH production in CKD were a decrease in serum calcium, a reduction in 1,25-di(OH)-vitamin D (calcitriol) synthesis by the kidney, and/or phosphate retention secondary to impaired renal excretion.

Since the cloning, more than a decade ago, of *klotho* and subsequently *fibroblast growth factor 23* (*FGF23*), their respective roles in calcium/phosphate metabolism have been progressively unraveled. (Note that the term 'klotho' corresponds, strictly speaking, to α-klotho, as subsequently another form of klotho, named β-klotho, has been identified.) Deletions of *klotho* and *FGF23* lead to similar phenotypes in the mouse, characterized by systemic features linked to accelerated aging, and by biochemical and morphologic features partially resembling those of the mineral and bone disorder of CKD (CKD-MBD). In particular, both $FGF23^{-/-}$ and $klotho^{-/-}$ mice develop hyperphosphatemia, vascular calcification, and osteopenia. In addition, plasma calcitriol is elevated, unlike what is usually observed in CKD-MBD except when high doses of active vitamin D derivatives are administered. It is clear at present that both klotho and FGF23 play an active role in the physiological control of mineral homeostasis and in the disturbances of calcium and phosphate metabolism characteristic of CKD-MBD.

On the basis of recent findings, FGF23 requires the presence of klotho in order to transform two of the four identified FGF receptors (FGFR1 through FGFR4) with low affinity for the hormone into receptors with high affinity, specifically subtypes IIIC of both FGFR1 and FGFR3. According to this theory, klotho functions as an obligatory co-receptor, although there are exceptions to this rule (see below). *Klotho* is expressed mainly in the kidney and the parathyroid gland, two organs with major importance for the regulation of calcium/phosphate metabolism. Its gene product is further expressed in the choroid plexus of the brain and, in addition, at very low levels in other tissues, including the liver, duodenum, pancreas, muscle, gonads, brain, and adipose tissue.

The main cooperative actions of FGF23 and klotho in the kidney consist of an inhibition of proximal tubular phosphate reabsorption (like the effect of PTH), and an inhibition of 25(OH)-vitamin D 1α-hydroxylase (opposite to the effect of PTH), via an interaction with FGFRs 1, 3, and/or 4. The specific FGFR involved in FGF23 and klotho interaction to inhibit tubular phosphate reabsorption remains a matter of debate. *klotho* is expressed only in the distal tubule. Colocalization of FGFR1 and klotho in the distal tubule suggests that this site may be an effector site of FGF23.[1] However, the main, if not exclusive, site of phosphate reabsorption in the kidney is the proximal tubule. This site expresses only FGFR3, not FGFR1, FGFR2, or FGFR4. One of the possible explanations for this apparent paradox is an intrarenal or extrarenal shuttle of the secreted extracellular form of klotho—probably as the full-length extracellular domain of its two internal repeat sequences, KL1 and KL2, detected in blood—and its subsequent binding to FGFR3 in the proximal tubule.

Interestingly, the KL1 and KL2 repeats of klotho each share amino acid sequence homology to family 1 glycosidases. The discovery of this homology paralleled the discovery of additional, probably FGF23-independent functions of klotho, including the regulation of the Ca^{2+} channel TRPV5 and the K^+ channel ROMK in the distal tubule, the Ca^{2+} channel TRPC6 in the heart, vascular smooth muscle and glomerular function; and of other processes such as insulin and insulin-like growth factor signaling and Na^+/K^+-ATPase activity.[2] More recently, it has been found that klotho appears to function as a sialidase rather than a glycosidase and thereby leads to an increase in the cell-surface abundance of these ion channels,

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via binding to galectin-1 as far as TRPV5 is concerned.[3] Interestingly, internalization of ROMK and that of TRPV5 occur via different pathways, the former by clathrin-coated vesicles, the latter by caveolae.[4,5]

The role of FGF23 and klotho in the parathyroid gland is more complex. Parathyroid tissue expresses the gene products of *FGFR1*, *FGFR3*, and *klotho*. FGF23 therefore could exert direct effects on the parathyroid, via cooperation with klotho at the receptor level as mentioned above. Studies have indeed shown a direct inhibitory effect of FGF23 on parathyroid gland FGFR signaling in animals *in vivo* and *ex vivo*,[6] and on PTH mRNA expression and protein secretion in bovine parathyroid cells *in vitro*.[7,8] The FGF23 effect in the latter might be direct or indirect, for example via stimulation of parathyroid 25(OH)-vitamin D 1α-hydroxylase, in contrast to the inhibitory FGF23 effect on the same enzyme in the kidney.

Klotho can directly modulate parathyroid Na^+/K^+-ATPase, independently of FGF23, via a molecular interaction with the α1-subunit of this enzyme, as shown by Imura *et al.*[9] This interaction supports the increase in the abundance of Na^+/K^+-ATPase at the plasma membrane, which is stimulated by low extracellular Ca^{2+}, but inhibited by high extracellular Ca^{2+} concentration. In *klotho*-deleted mice the Na^+/K^+-ATPase-dependent stimulation of PTH secretion by low extracellular Ca^{2+} is negligible, compared with that in *klotho*-sufficient wild-type mice. According to the authors' hypothesis, the resulting enhancement of Na^+/K^+-ATPase activity could create an electrochemical gradient that in turn would enhance PTH secretion. Alternatively, enhanced Na efflux could modify transepithelial Ca^{2+} transport via the Na^+/Ca^{2+} exchanger and thereby modify PTH secretion.

Somewhat surprisingly, patients with advanced stages of CKD generally have markedly increased serum FGF23 levels, together with high, not low, serum PTH levels. There is actually abundant clinical and experimental evidence in favor of a positive association of circulating FGF23 with PTH. In CKD, this could be attributed to chronic hyperphosphatemia, which has been shown to stimulate synthesis of both FGF23 by the bone and PTH by the parathyroid gland, via direct and indirect mechanisms. Moreover, the renal excretion of FGF23 could be impaired, leading to further increase in the serum. Treatment with active vitamin D derivatives also could explain high serum FGF23 levels, as calcitriol directly stimulates skeletal FGF23 secretion, together with persisting high serum PTH levels due to parathyroid resistance to the action of vitamin D sterols.

Mice overexpressing FGF23, with initially normal kidney function, exhibit high serum PTH levels and parathyroid hyperplasia. This association has been attributed to the inhibitory effect of FGF23 on renal 25(OH)-vitamin D 1α-hydroxylase and the ensuing decrease in circulating calcitriol, which leads to a decrease in intestinal calcium and phosphate absorption, a decrease in serum calcium, and thereby an increase in PTH secretion.

Another possibility could be a direct or indirect stimulation by hyperparathyroidism of FGF23 synthesis in the bone, as suggested by two observations of high serum FGF23 levels in association with hyperparathyroidism or pseudohyperparathyroidism of nonrenal origin.[10,11] The first patient had a chromosomal translocation with increased serum klotho and PTH levels. The second had Jansen's disease with constitutively activated PTH receptor 1 mimicking primary hyperparathyroidism.

An interesting novel explanation for the absence of an inverse relation between serum FGF23 and PTH levels in patients with CKD has been provided by Komaba *et al.*[12] These authors observed a decrease in klotho and FGFR1 immunoreactivity and mRNA expression in parathyroid tissue of chronic hemodialysis patients, particularly in nodular areas of the hyperplastic glands. In contrast, the expression of Ki67, a marker of cell proliferation, was upregulated. On the basis of their findings, the authors postulated the existence of relative parathyroid gland resistance to FGF23. Galitzer *et al.*[13] provided experimental support for this postulate. They found a decrease in parathyroid mRNA and protein expression of both klotho and FGFR1 in rats with chronic renal failure. Moreover, recombinant FGF23 failed to reduce serum PTH or to activate mitogen-activated protein kinase signaling in the parathyroid glands of uremic rats *in vivo*, and to inhibit PTH mRNA expression and PTH secretion by uremic rat parathyroid gland tissue *in vitro*, in contrast to the expected inhibitory response in normal rat parathyroids.

Hofman-Bang *et al.*[14] (this issue) now report the unexpected finding of an increase, not a decrease, in the gene expression of *klotho*, *FGFR1IIIC*, and *Na^+/K^+-ATPase* in the parathyroid glands of rats with chronic renal failure. Their observation appears to be in conflict with those of Galitzer *et al.*,[13] Komaba *et al.*,[12] and Kumata *et al.*,[15] although in agreement with that of Ohkido *et al.*[16] The finding by Hofman-Bang *et al.*[14] is counterintuitive, since in presence of persistently high serum PTH levels in CKD one would expect at least some degree of resistance to the action of FGF23, similar to the relative resistance of the parathyroid to extracellular Ca^{2+} and calcitriol.

Hofman-Bang *et al.*[14] point out that in animal models the expression of *klotho* and *FGFR1* in the parathyroid actually has been found to be variable, depending on the stage of CKD and the duration of the uremic state. In the study by Galitzer *et al.*,[13] parathyroid *klotho* expression was indeed significantly increased after 2 weeks of uremia but was subsequently suppressed at 6 weeks of uremia, whereas *FGFR1* expression was decreased at both 2 and 6 weeks.

What other factors could explain these discrepant results? Findings in experimental animals are not necessarily the same as those in human patients. Concerning the discrepancy between the findings of their animal study and that of Galitzer *et al.*,[13] Hofman-Bang *et al.*[14] discuss differences in experimental models, in particular a different mode of CKD induction, as well as possible differences in diet composition. The markedly higher serum creatinine in the uremic rats of the study by Galitzer *et al.*[13] could be part of the explanation. In line with this theory, dialysis patients with the most severe CKD and secondary hyperparathyroidism had an overall decrease in klotho expression, according to Komaba *et al.*[12] and Kumata *et al.*,[15] although the degree of

suppression also appeared to depend on the type of parathyroid-cell proliferation. Nodular, probably clonally growing areas of parathyroid tissue exhibited lower klotho and FGFR1 expression than diffusely hyperplastic areas.[12]

There are other possible explanations. We do not have precise information on vitamin D status in these animal studies. Low serum calcitriol levels might favor high *klotho* expression. In the work by Hofman-Bang *et al.*,[14] parathyroid klotho expression was reduced by calcitriol given to uremic rats on a high-phosphate diet. This reduction was associated with a further increase in serum phosphate, rendering a major stimulatory role of high phosphate improbable *ipso facto*.

As for FGFR expression, FGFR1 IIIC was upregulated but FGFR3 IIIC was unchanged in the uremic rats of Hofman-Bang *et al.*[14] In addition to differing ligand affinities and tissue distribution, the regulation of FGFR subtype expression and post-receptor signaling might differ, depending on specific disturbances of calcium and phosphate metabolism.

Finally, Hofman-Bang *et al.*[14] make the attractive hypothesis that the observed upregulation of klotho could be part of an Na^+/K^+-ATPase-driven mechanism aimed at increasing PTH secretion in response to hypocalcemia. According to this scenario, increased expression of klotho in early stages of CKD could allow an increase in its binding to the α1-subunit of the enzyme and its subsequent enhanced translocation to the parathyroid-cell membrane. This in turn would enhance Na^+/K^+-ATPase activity and favor increased PTH secretion (see above). This mechanism would be countered by the upregulation of FGFR1 IIIC together with its co-receptor klotho, favoring a decrease in PTH secretion in response to FGF23. In later stages of CKD the opposite would occur, as a result of downregulation of both klotho and FGFR expression. This hypothetical scenario could be called a yin–yang situation, as schematically illustrated in Figure 1. Thus in the yin situation enhanced PTH secretion would mainly be due to a fully active klotho–Na^+/K^+-ATPase interaction pathway despite concomitant inhibition via the FGFR pathway. In contrast, in the yang situation the normal inhibition of PTH secretion via the FGFR pathway would not work well or not work at all in case of klotho downregulation, and this stimulation of the parathyroid would not be overcome by a lower activity of the klotho–Na^+/K^+-ATPase pathway. It might explain why secondary hyperparathyroidism evolves unabated in CKD—because of ongoing stimulation of PTH synthesis and secretion and only partially efficient counterregulatory mechanisms.

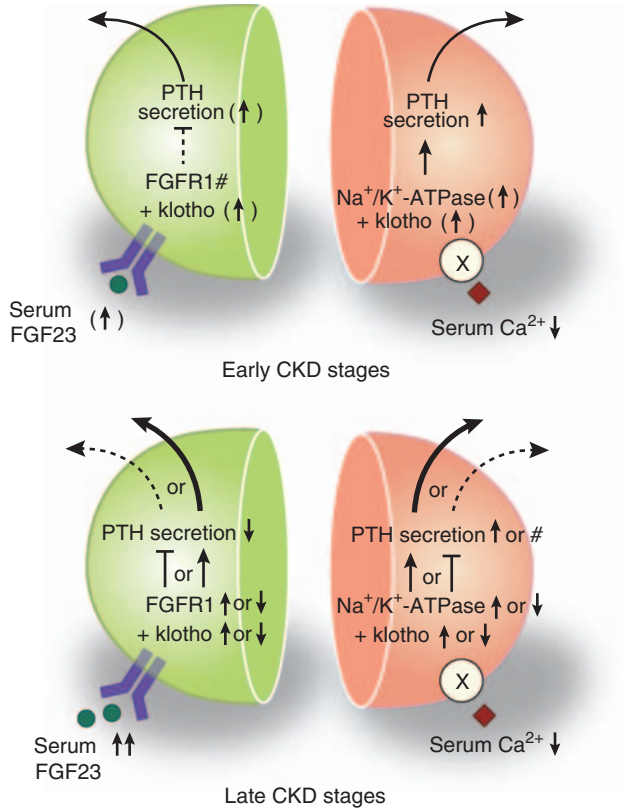

Figure 1 | Potential mechanisms of persistent PTH oversecretion in CKD involving the FGF23-klotho system. An increase in expression of klotho in early stages of CKD could allow its enhanced binding to the α1-subunit of the Na^+/K^+-ATPase enzyme and an increase in its abundance in the parathyroid-cell membrane. This in turn would enhance Na^+/K^+-ATPase activity by the mechanism outlined in the manuscript. Upregulation of klotho, favoring an increase in PTH secretion via the Na^+/K^+-ATPase pathway, would be countered to some degree by upregulation of FGFR1 IIIC together with its co-receptor klotho, favoring a decrease in PTH secretion in response to FGF23. In later stages of CKD, the opposite scenario would occur, as a result of downregulation of both klotho and FGFR expression. #, no change.

DISCLOSURE

The author declared no competing interests.

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Tenofovir-induced kidney disease: an acquired renal tubular mitochondriopathy

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Tenofovir, used in combination with other antiretroviral agents, is an effective therapy for HIV infection. Although large clinical studies and post-marketing data support a benign renal profile for tenofovir, numerous cases of kidney injury raise concern for nephrotoxic potential. Early human studies and experimental evidence suggested that tenofovir itself was not associated with mitochondrial toxicity within the kidney. However, recent animal data demonstrate that tenofovir causes mitochondrial DNA depletion and mitochondrial toxicity. Herlitz *et al.* confirm the nephrotoxicity of tenofovir in humans. They describe its clinical consequences, histopathologic findings, and its mitochondrial toxicity in HIV$^+$ patients.

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Drug research and development facilitates the synthesis and release of novel therapeutics into clinical practice at a remarkable rate. Their availability has undoubtedly revolutionized the treatment of numerous diseases—in particular, devastating processes such as HIV infection. The landscape of this disease has been forever changed by the availability of highly active antiretroviral therapy (HAART) since 1996. The basic premise behind this strategy is to attack the virus at different replication steps using multiple medications. Unfortunately, most success stories have a catch; in this case, the price of success is drug-induced kidney disease, a challenging complication for HIV$^+$ patients and their caregivers.[1]

Before the widespread use of HAART, HIV-associated kidney disease was primarily due to direct and/or indirect effects of the virus. However, with successful treatment of HIV infection, drug-induced nephrotoxicity (Table 1) surfaced as an important problem. Acute kidney injury (AKI), various tubulopathies, nephrolithiasis, and chronic kidney disease were noted.[1] HAART causes these renal syndromes via multiple mechanisms, including direct tubular toxicity, allergic reactions, and precipitation of insoluble drug crystals.

Although many of the early drugs had intolerable adverse effects and difficult dosing schedules, newer agents have overcome many of these issues. One such drug, tenofovir, has gained widespread use on the basis of its efficacy, tolerability, and patient-friendly dosing schedule.[2] As it is structurally similar to the acyclic nucleotide analogs adefovir and cidofovir, which are nephrotoxic, concern about adverse renal effects existed for tenofovir as well. These two drugs cause proximal tubulopathies such as AKI as a result of acute tubular necrosis (ATN) and Fanconi's syndrome,[3] by disrupting proximal tubular mitochondrial function. A number of mechanisms underlie drug-induced mitochondriopathies, but these drugs act primarily by decreasing mitochondrial DNA (mtDNA) replication by inhibiting mitochondrial DNA polymerase-γ, which is the only enzyme capable of replicating mtDNA.[3] As a result, mtDNA and a number of the mtDNA-encoded enzymes involved in electron transport chain function and oxidative phosphorylation are depleted, resulting in disturbed mitochondrial function. This ultimately causes, among other effects, a deficit in adenosine triphosphate production, impaired cell function, and cell injury and/or death.

Early randomized clinical trials[4] and post-marketing data[5] examining tenofovir in relatively healthy HIV$^+$ subjects supported an excellent safety profile, including the absence of significant renal injury. An *in vitro* experimental study supported this clinical finding.[6] The investigators exposed various cultured human cell lines (liver, muscle, proximal renal tubule) to tenofovir. Minimal mtDNA depletion and insignificant reductions in cellular expression of the mitochondrial protein cytochrome *c* oxidase were noted with tenofovir. However, with the release of tenofovir into clinical practice and its use in HIV patients with various comorbid conditions, reports of nephrotoxicity began to surface.[7] These reports described

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